

Title: TARGET-DEPENDENT TRANSCRIPTION USING
DELETION MUTANTS OF N4 RNA POLYMERASE

Inventor(s): Elena K. Davydova/Lucia B. Rothman-Denes/Gary A. Dahl/
Svetlana Y. Gerdes/Jerome J. Jendrisak

Application No.:

Docket Number: 310307.00006

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Bacteriophage N4 vRNAP promoters

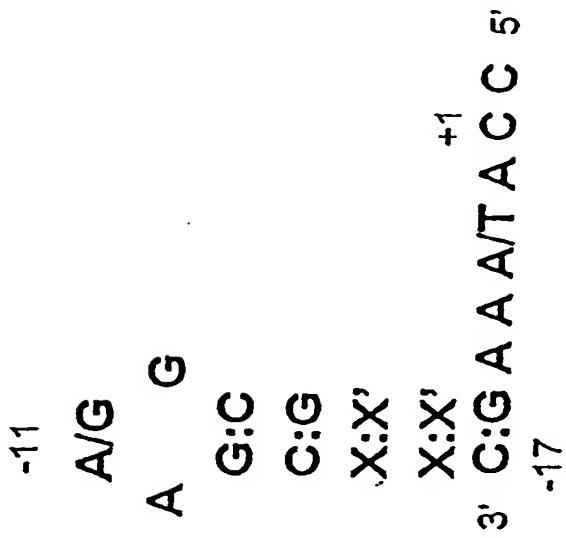


FIG. 1

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N4 vRNAP and generation of mini-vRNAP

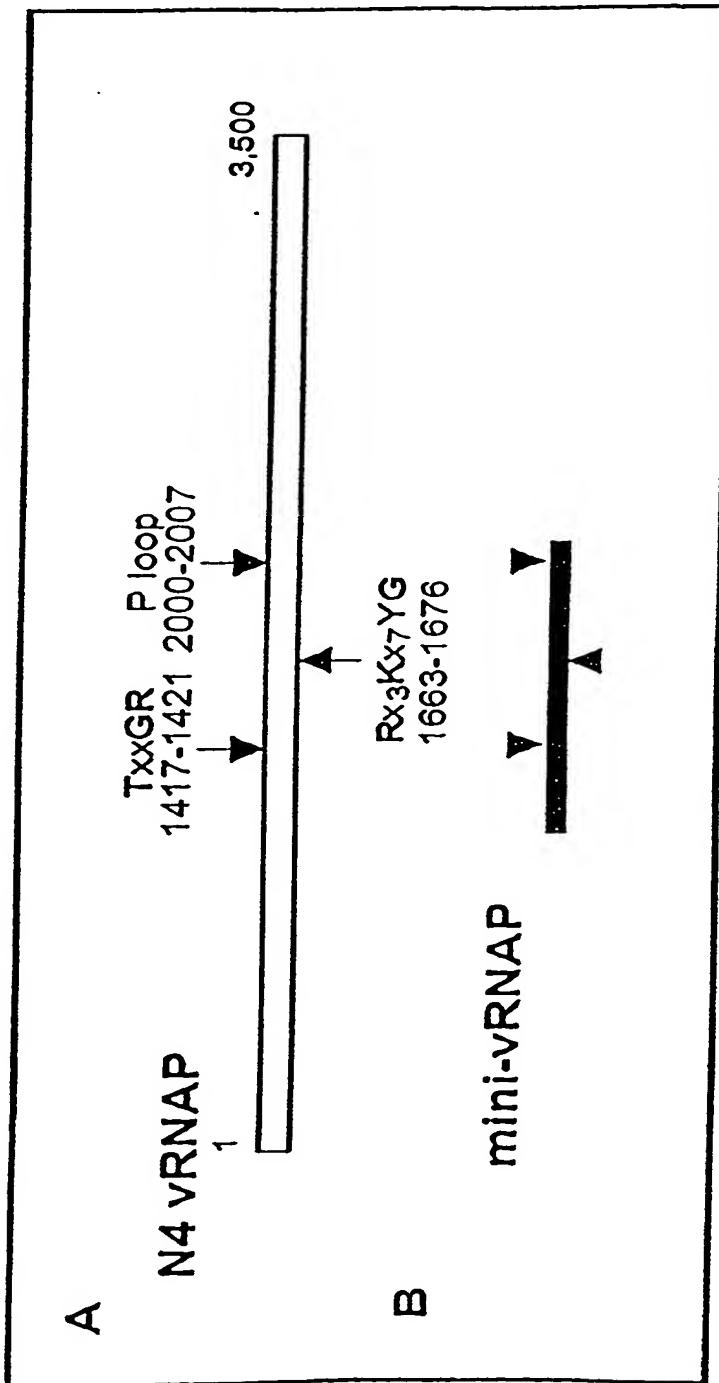


FIG. 2

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Identification of the minimal active domain of N4 vRNAP
by proteolytic cleavage.

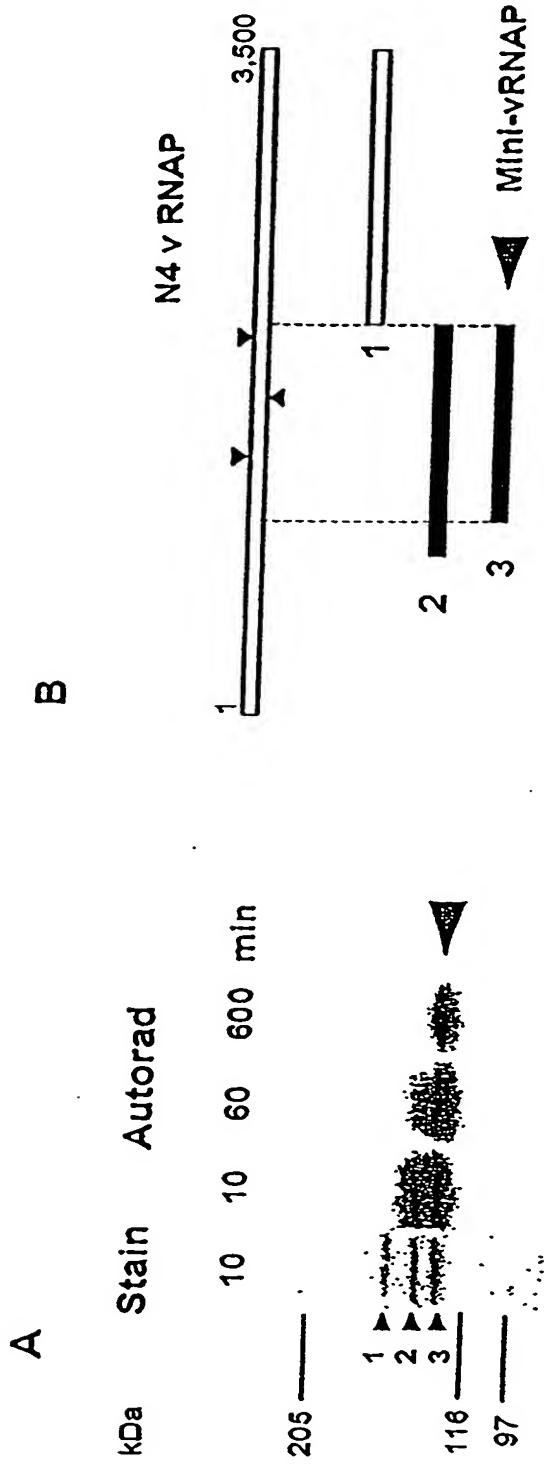


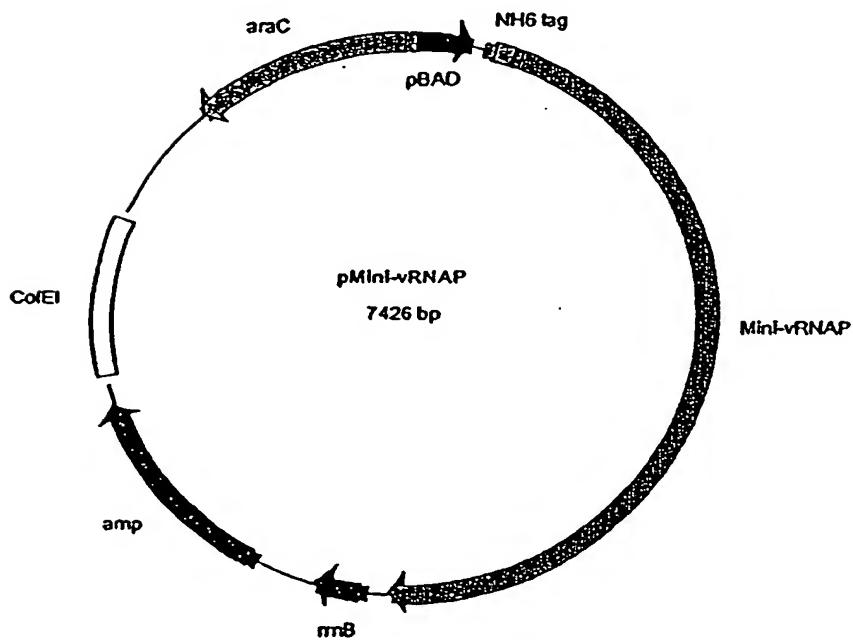
FIG. 3

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Plasmid name: pMini-vRNAP
Plasmid size: 7426 bp
Constructed by: K. M. Kazmierczak
Construction date: 2/2000
Comments: Insert cloned into Invitrogen
pBAD B expression plasmid

FIG 4

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Purification of cloned vRNAP and mini-vRNAP

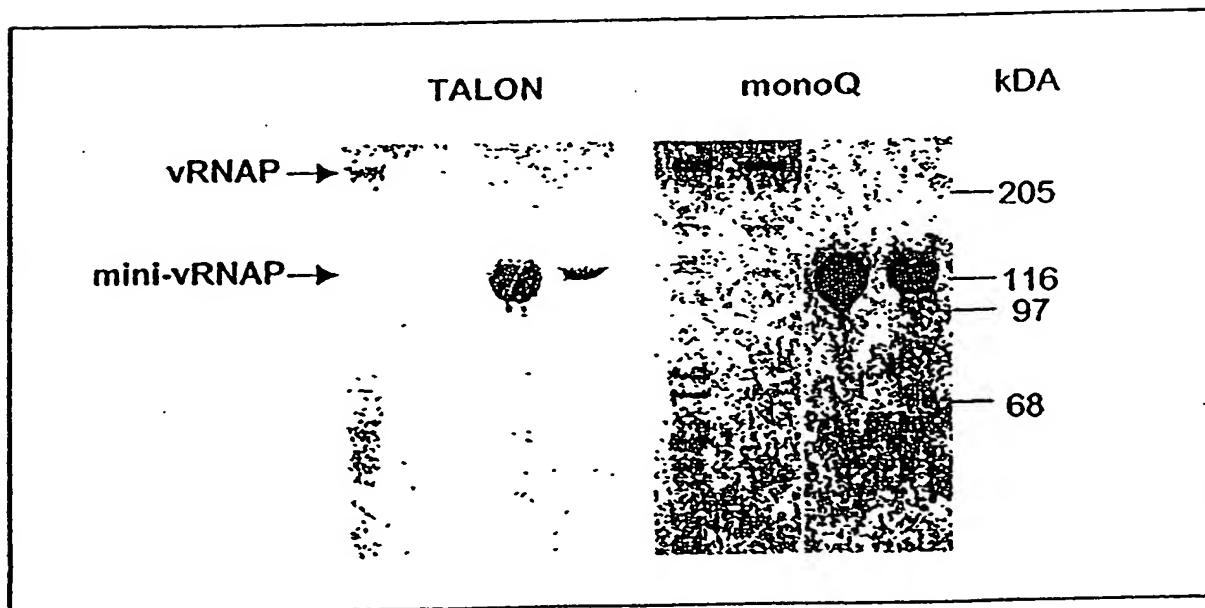


FIG 5

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Activation of N4 vRNAP transcription by *Eco* SSB at different ssDNA concentrations

ssDNA, nM	0.8	4	20			
<i>Eco</i> SSB	+	-	+	-	+	-

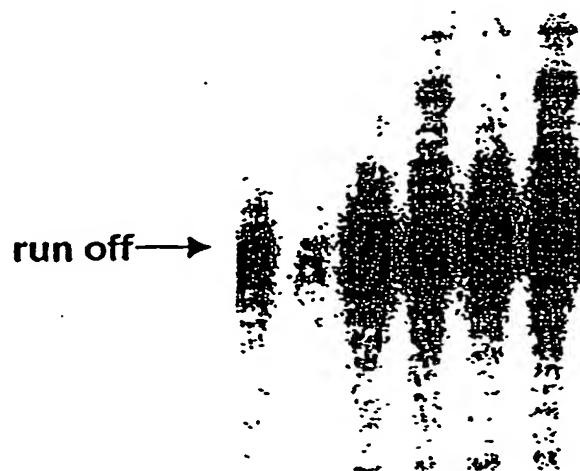


FIG 6

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Effect of *Eco* SSB on ssDNA template recycling

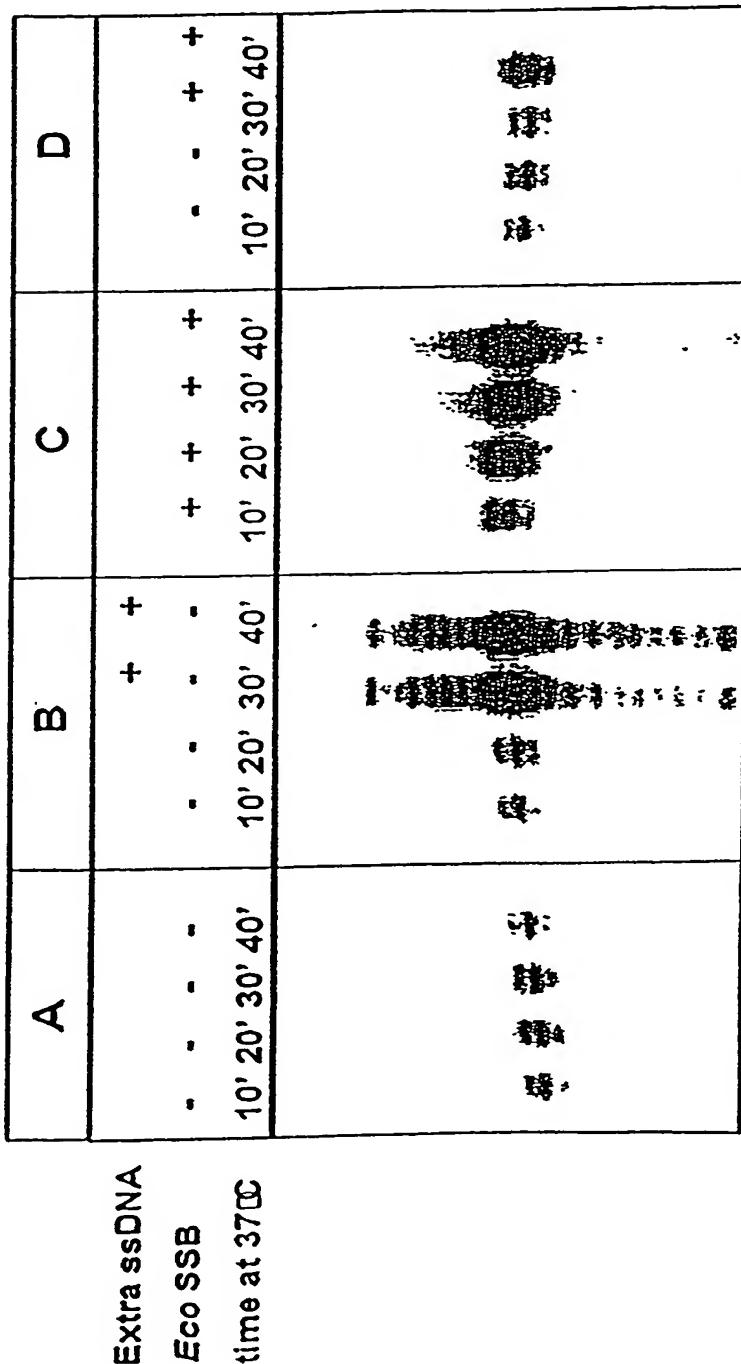


FIG. 7

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Effect of *Eco SSB* on the state of template DNA
and product RNA in vRNAP transcription

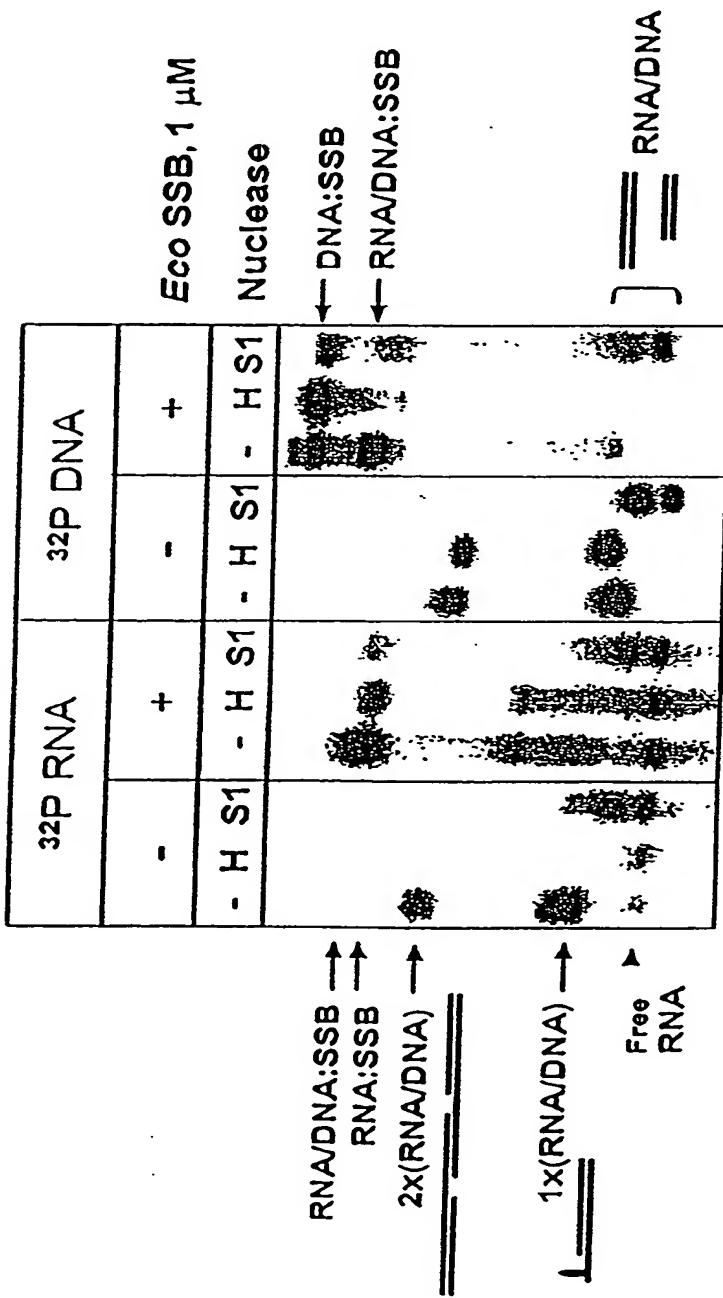


FIG. 8

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Transcription initiation by vRNAP and mini-vRNAP

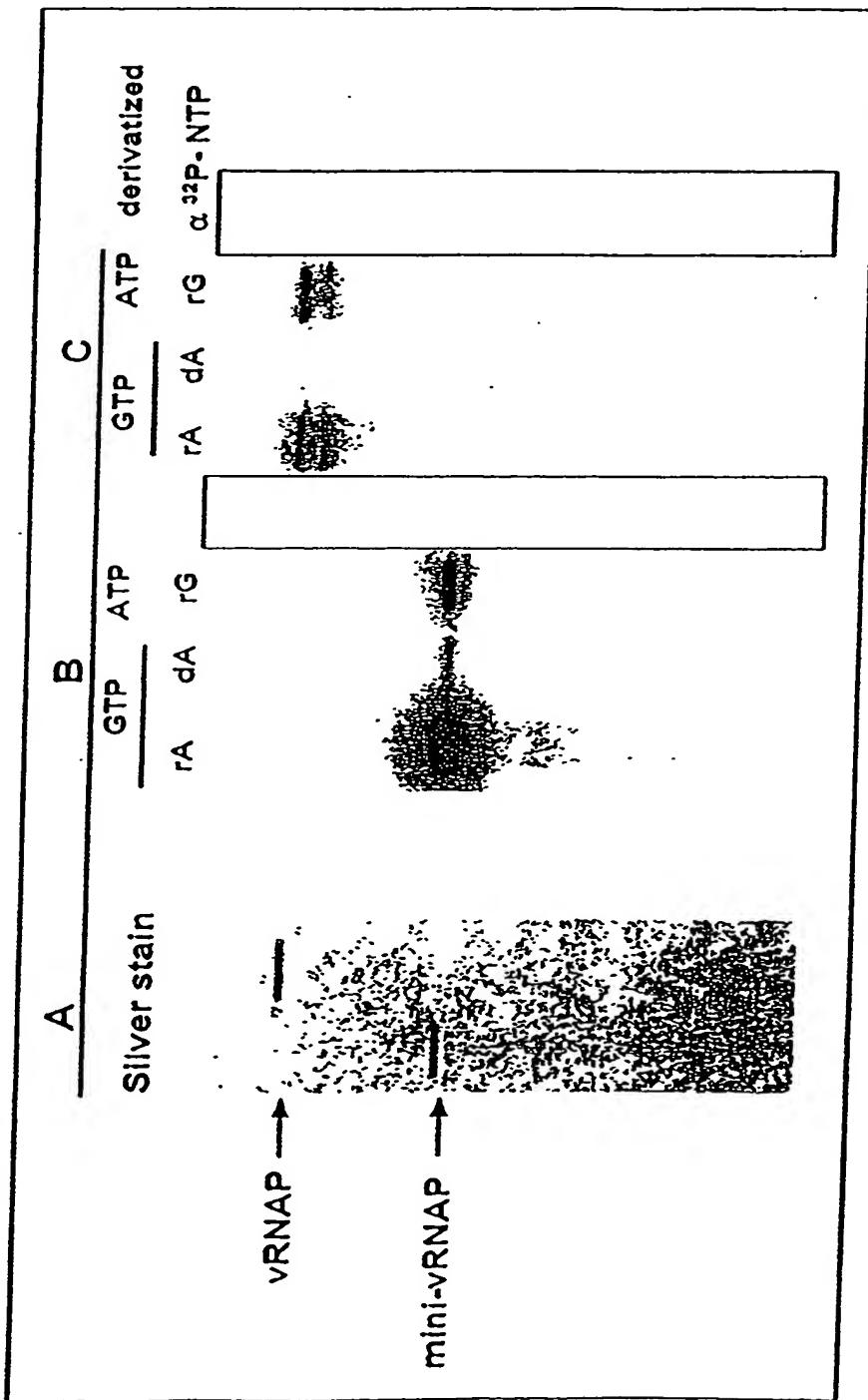


FIG. 9

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Effect of *Eco* SSB on transcription of vRNAP and mini-RNAP

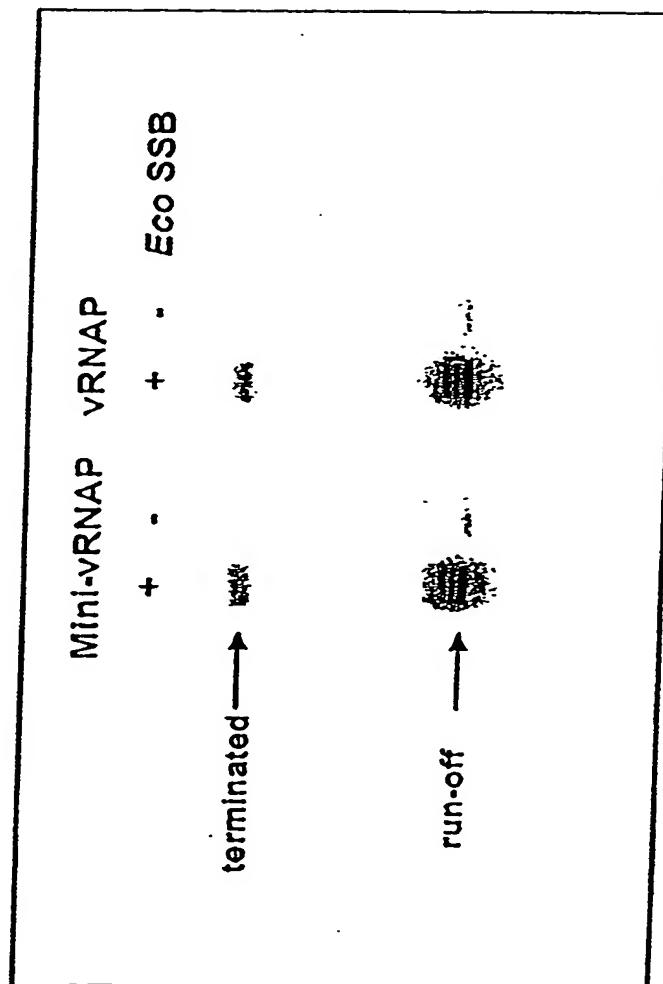


FIG. 10

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Determination of mini-vRNAP promoter contacts

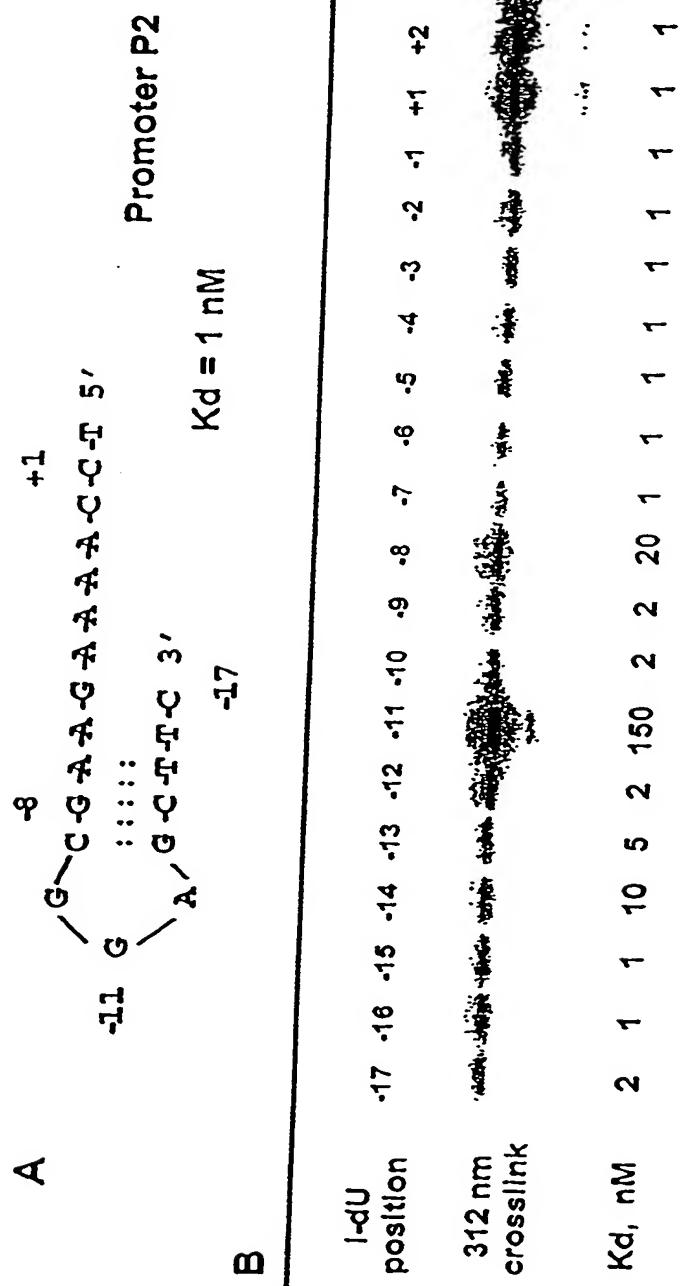


FIG. 11

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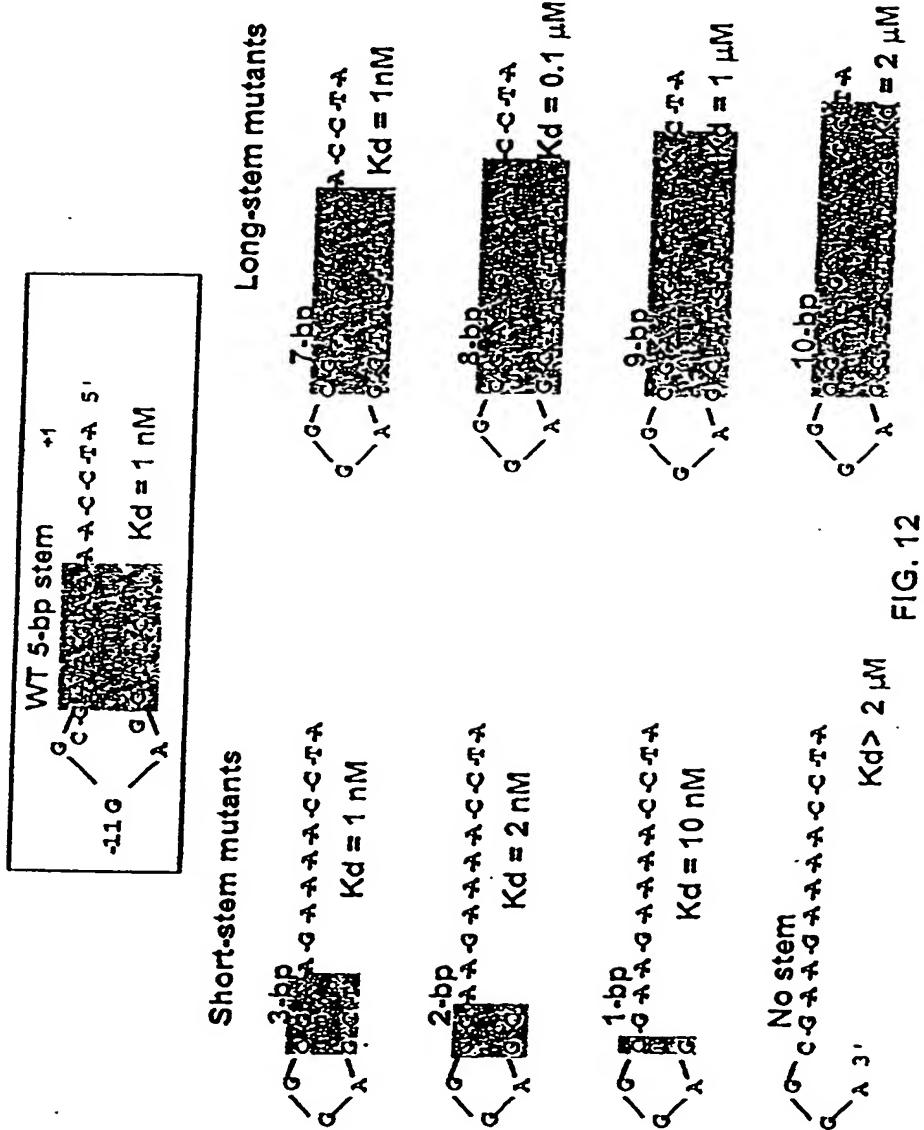
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Binding affinities of stem-length promoter mutants



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Identification of the transcription start site by catalytic
autolabeling

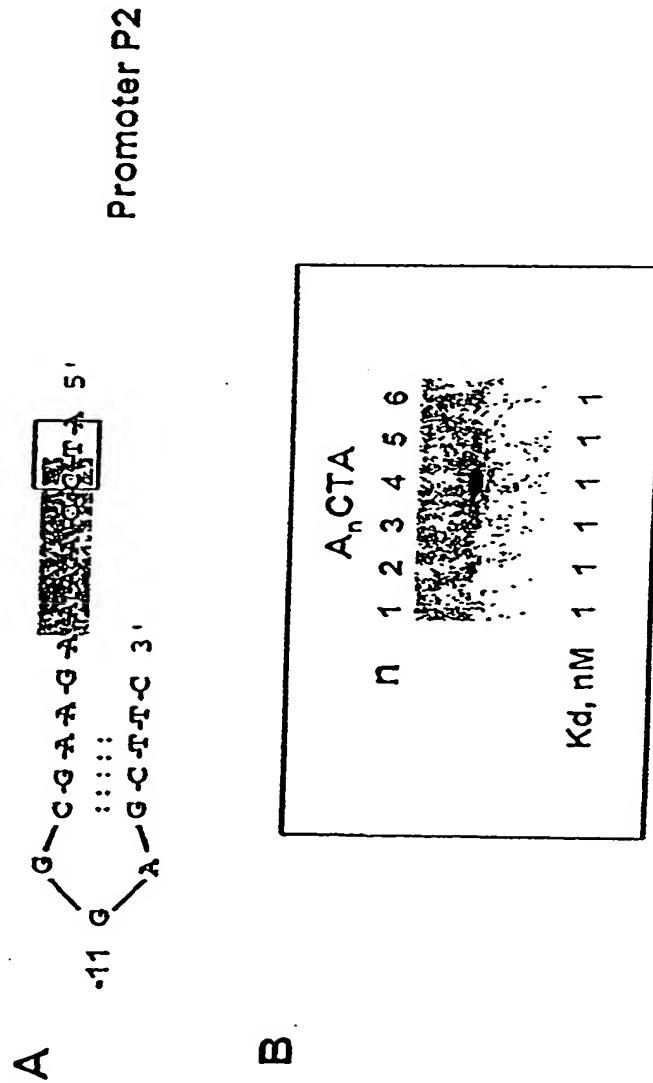


FIG. 13

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UV crosslinking of mutant mini-vRNAPases
to promoter oligonucleotides

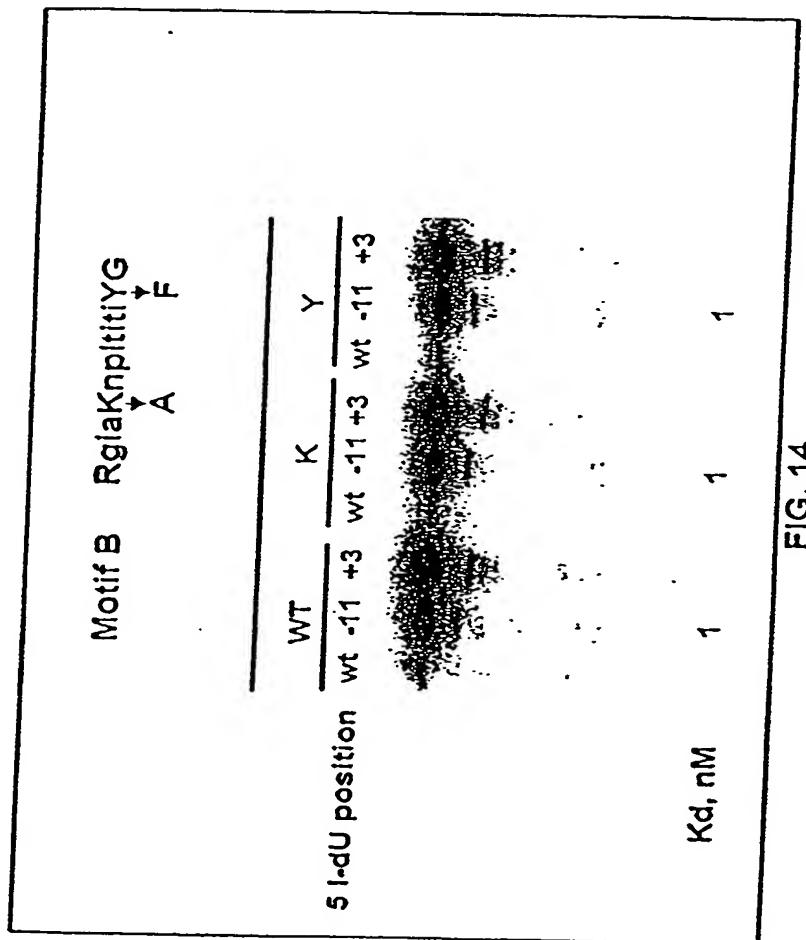


FIG. 14

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Run-off transcription by mutant mini-vRNAPases

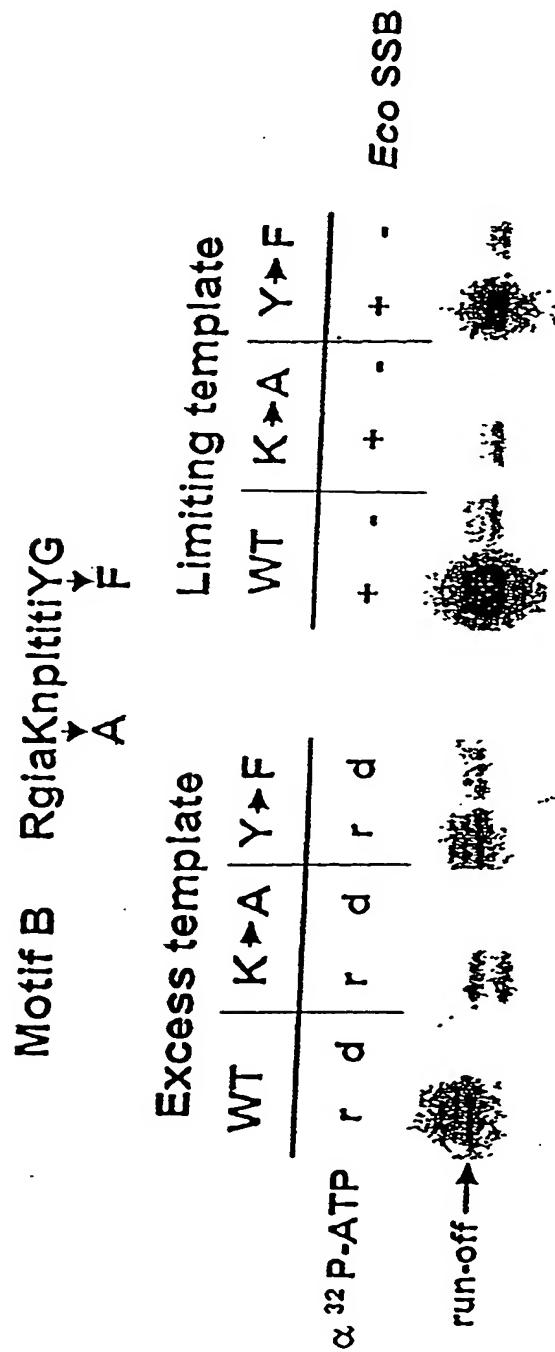


FIG. 15

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Mutant mini-vRNAPases transcription initiation

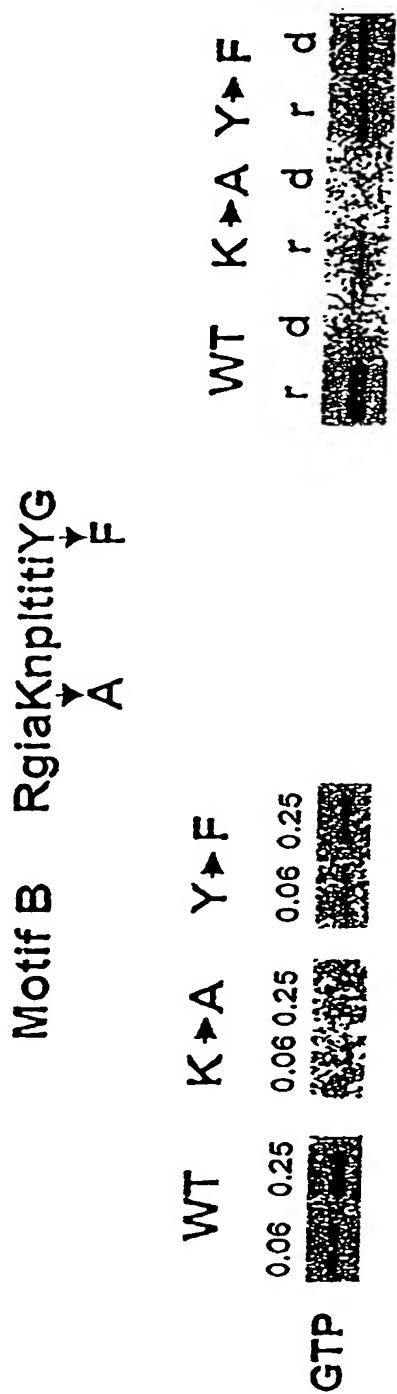


FIG. 16

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Detection of *in vivo* activities of N4 vRNAP and mini-vRNAP

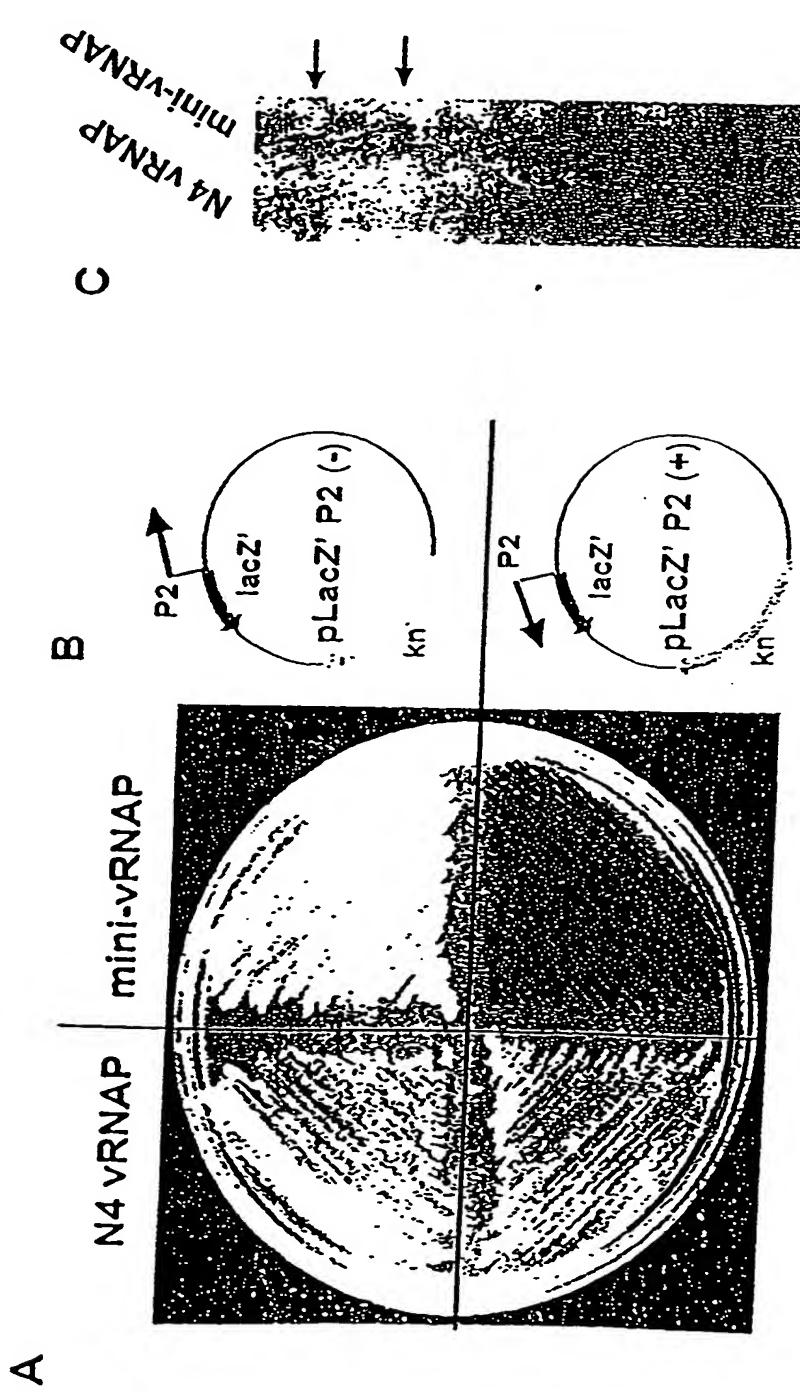


FIG. 17

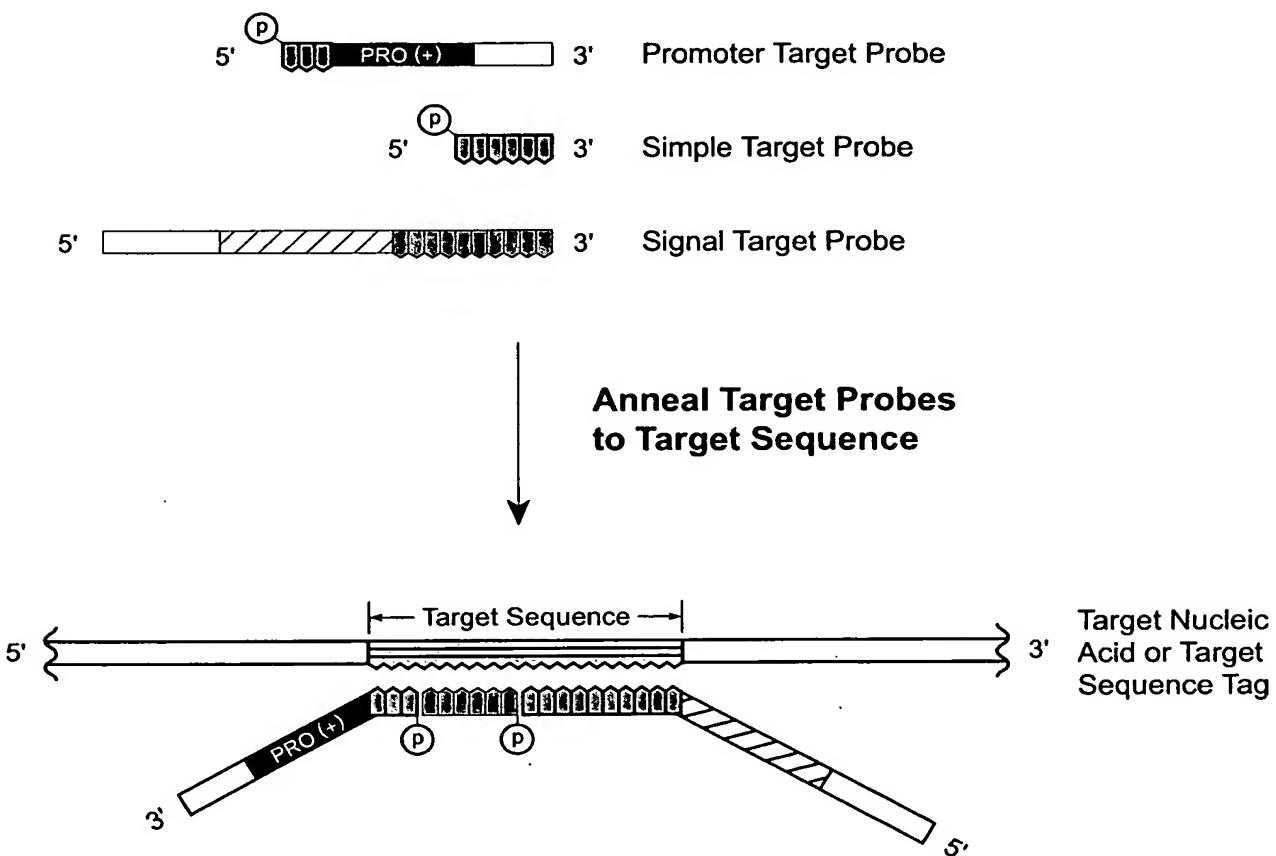
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Monopartite Target Probes



Portions of a Monopartite Target Probe

Single-Stranded Transcription Promoter

Target-Complementary Sequence

Signal Sequence

Optional Sequences

(p)- Phosphate Group

FIG 18

Title: TARGET-DEPENDENT TRANSCRIPTION USING
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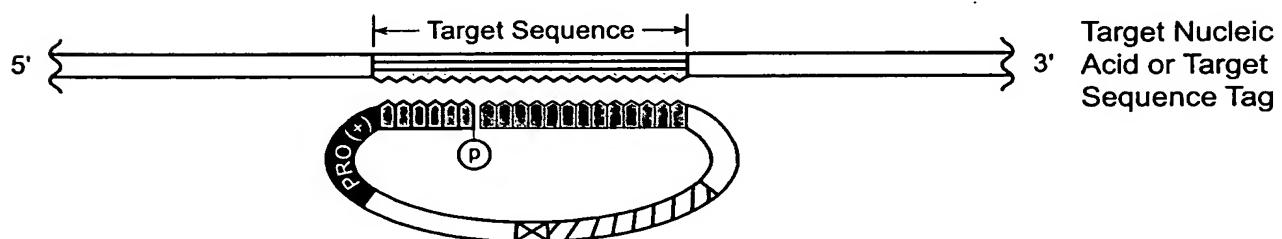
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Anneal Bipartite Target Probe
to Target Sequence



Portions of a Bipartite Target Probe

PRO (+) Single-Stranded Transcription Promoter

Target-Complementary Sequence

Signal Sequence

Transcription Termination Sequence(s)

Optional Sequences

Phosphate Group

FIG 19

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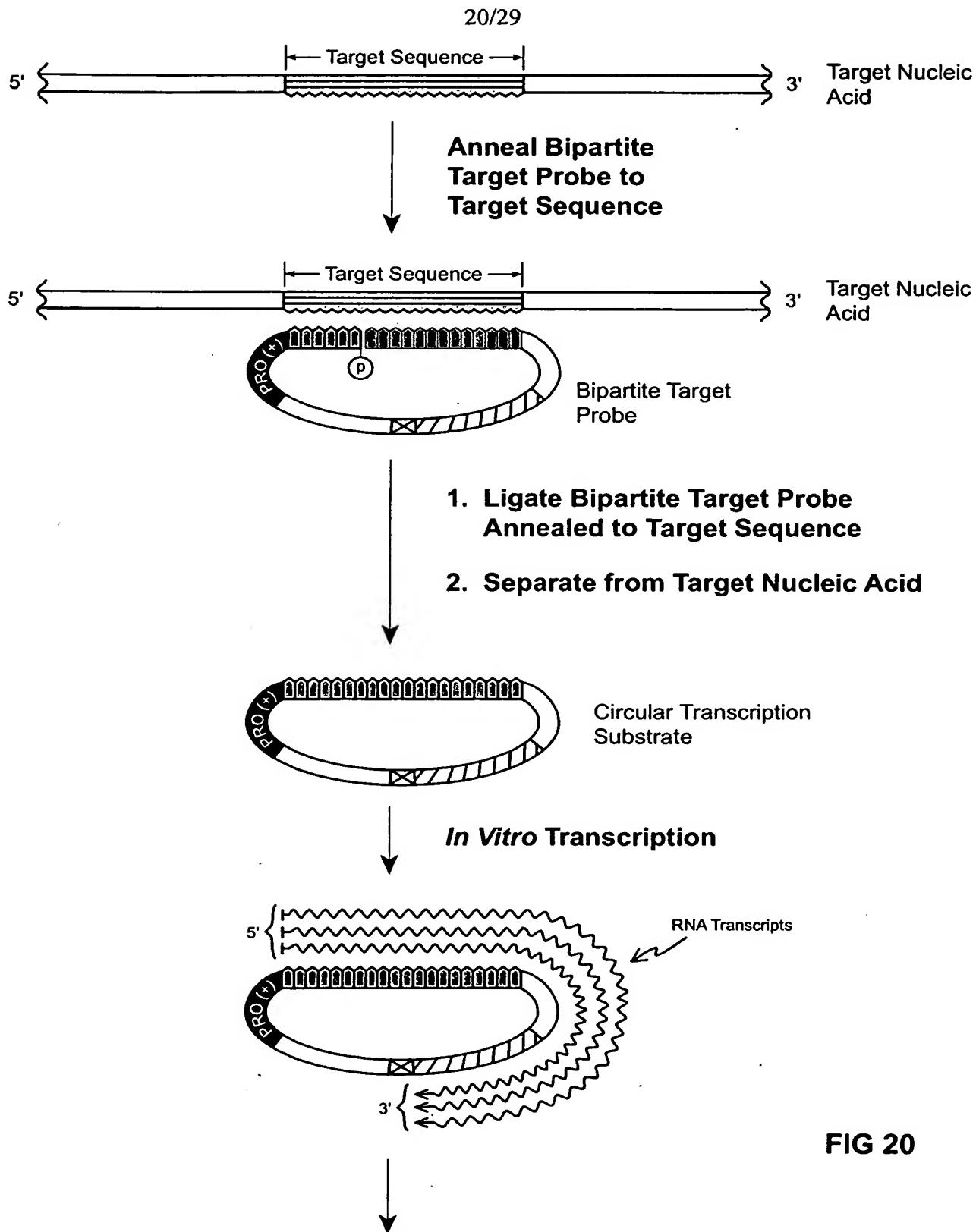


FIG 20

Detect RNA Transcription Products Directly or Indirectly

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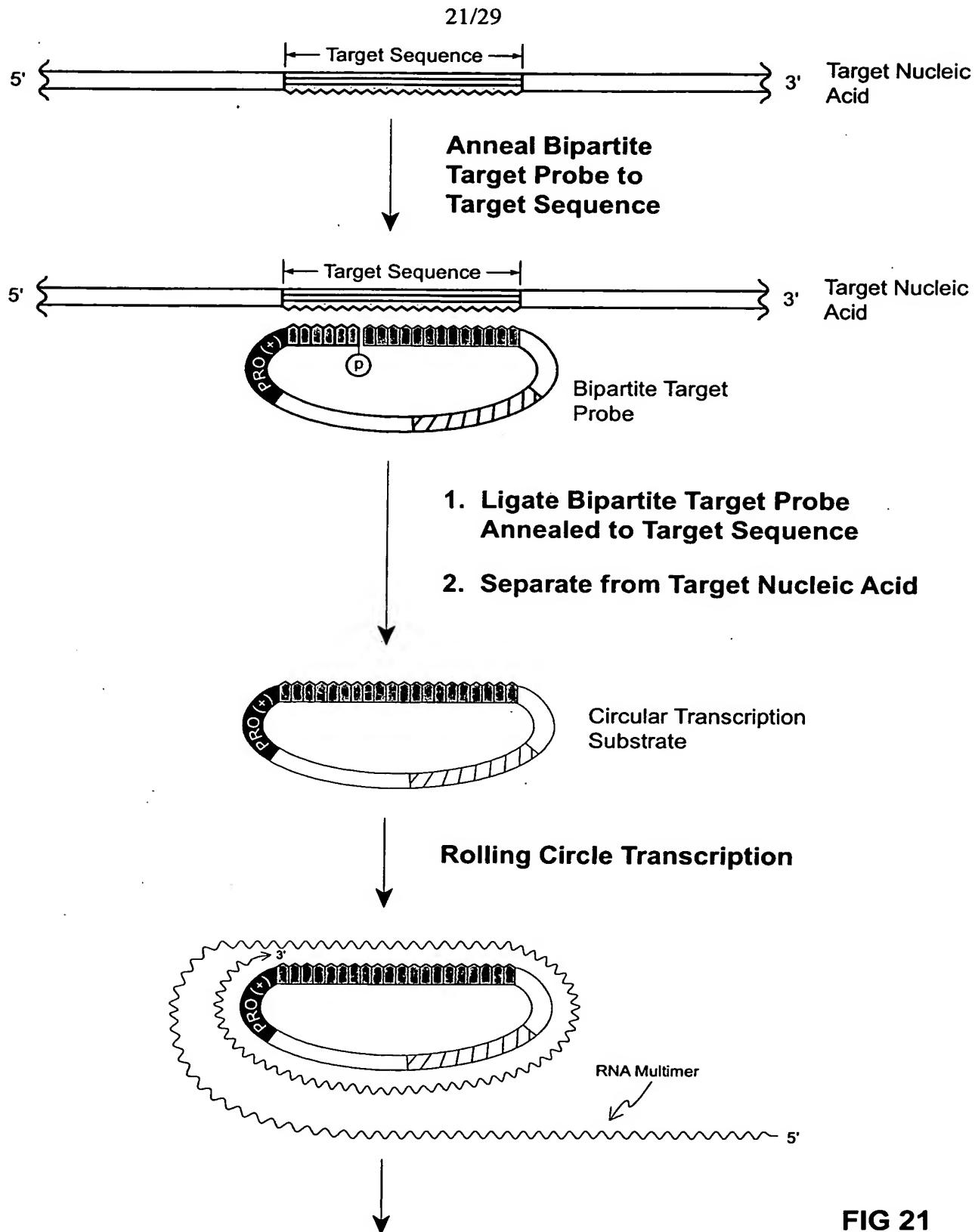


FIG 21

Detect RNA Transcription Products Directly or Indirectly

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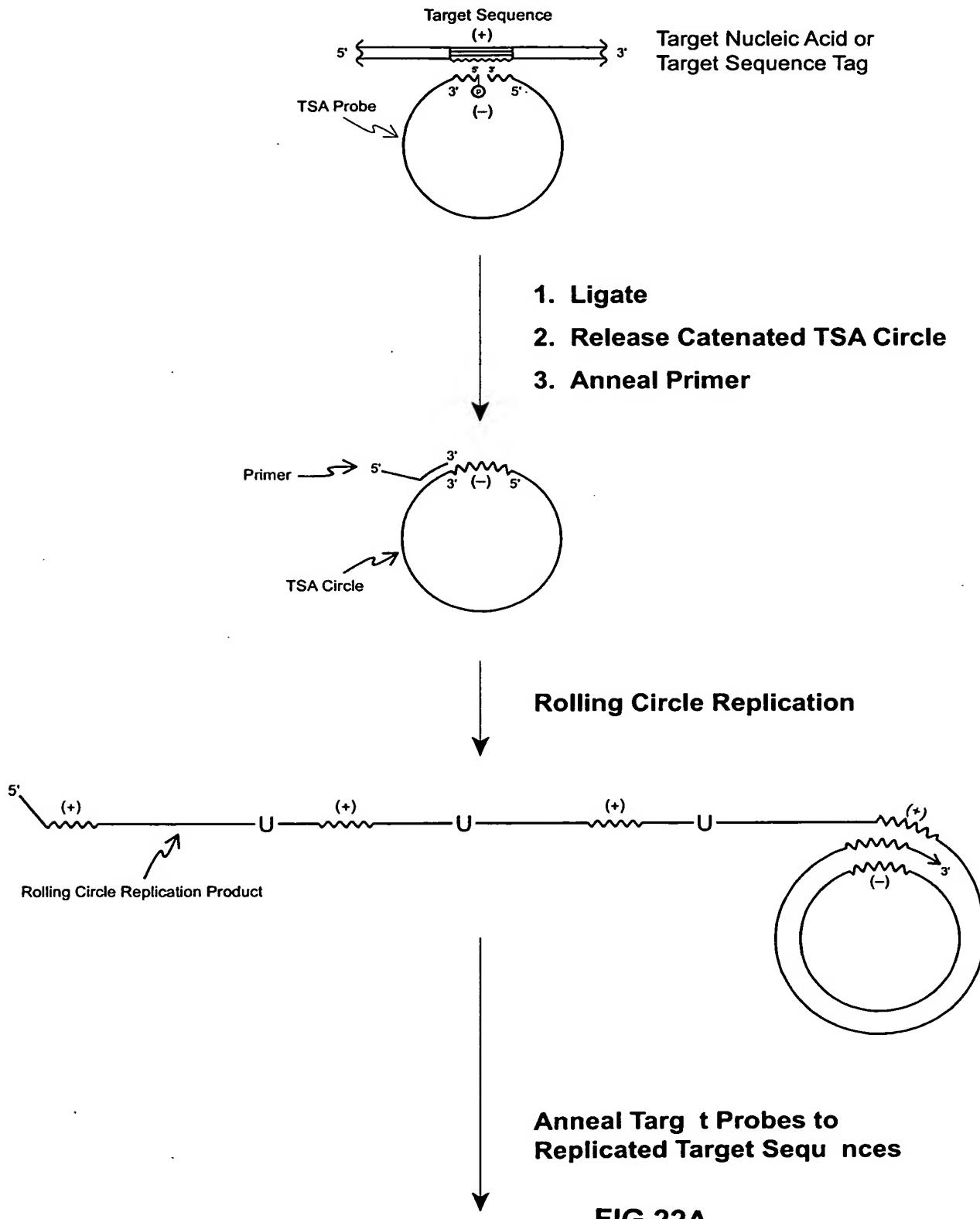
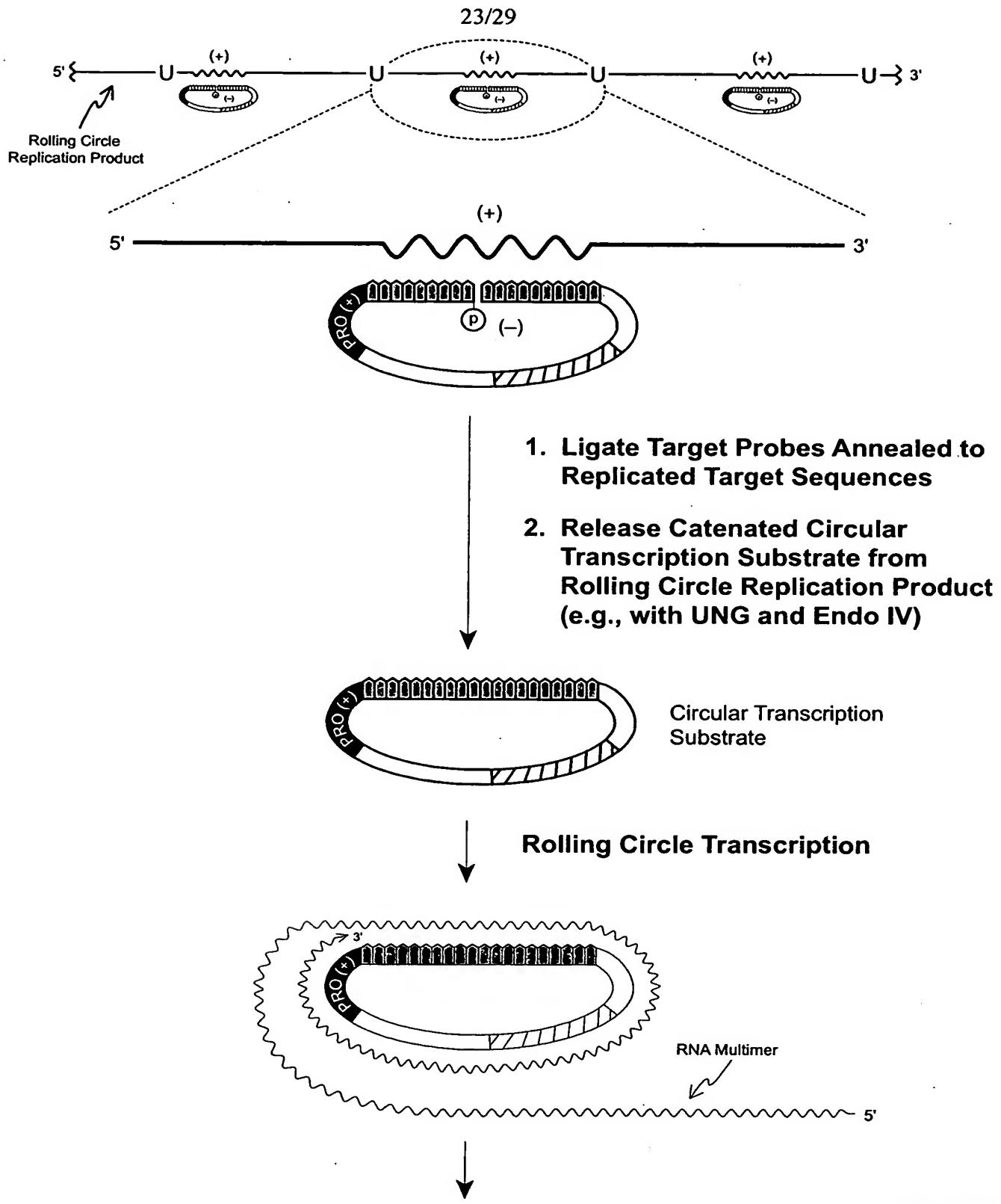


FIG 22A

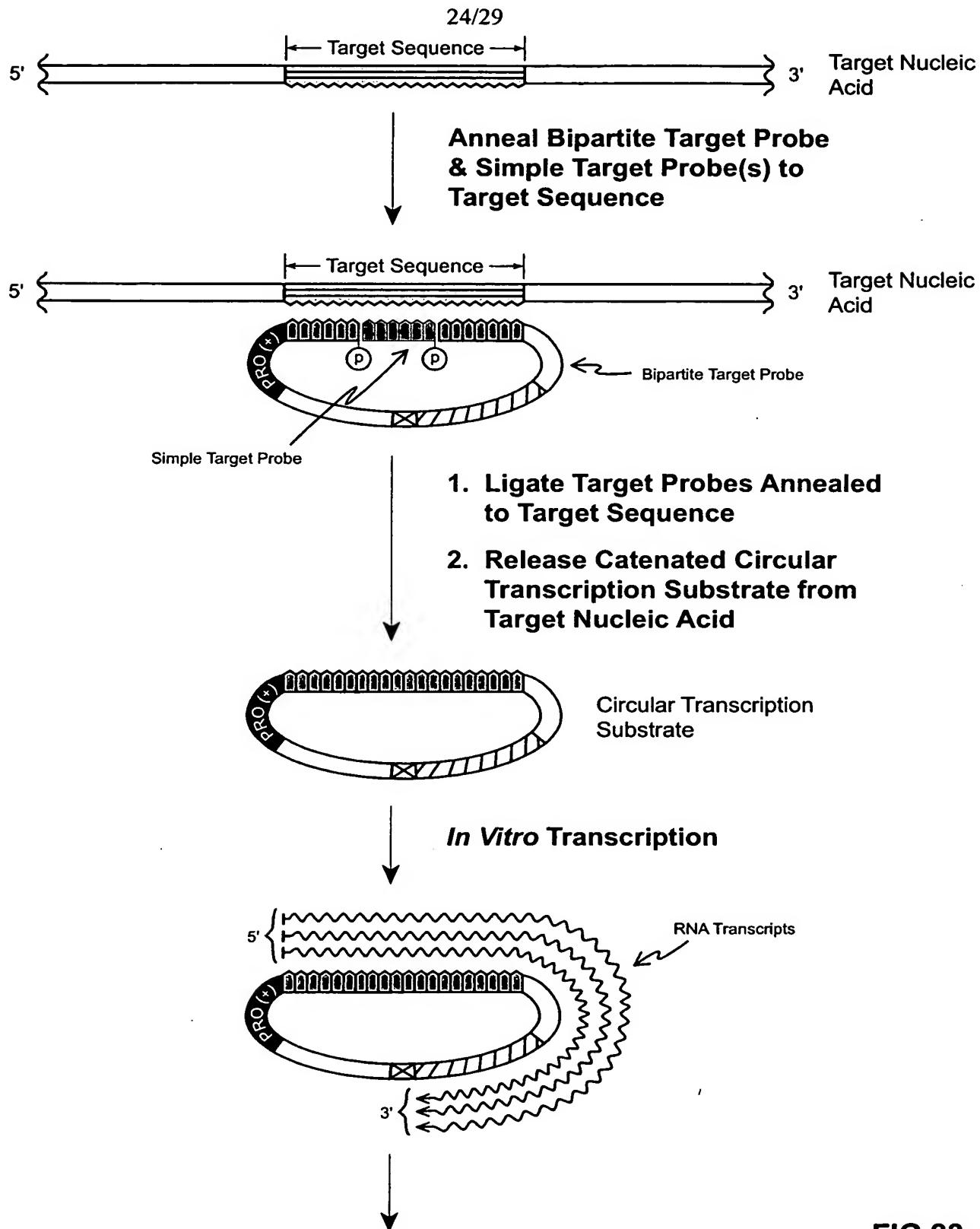
Title: TARGET-DEPENDENT TRANSCRIPTION USING
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Detect RNA Transcription Products Directly or Indirectly

FIG 22B

Title: TARGET-DEPENDENT TRANSCRIPTION USING
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Detect RNA Transcription Products Directly or Indirectly

FIG 23

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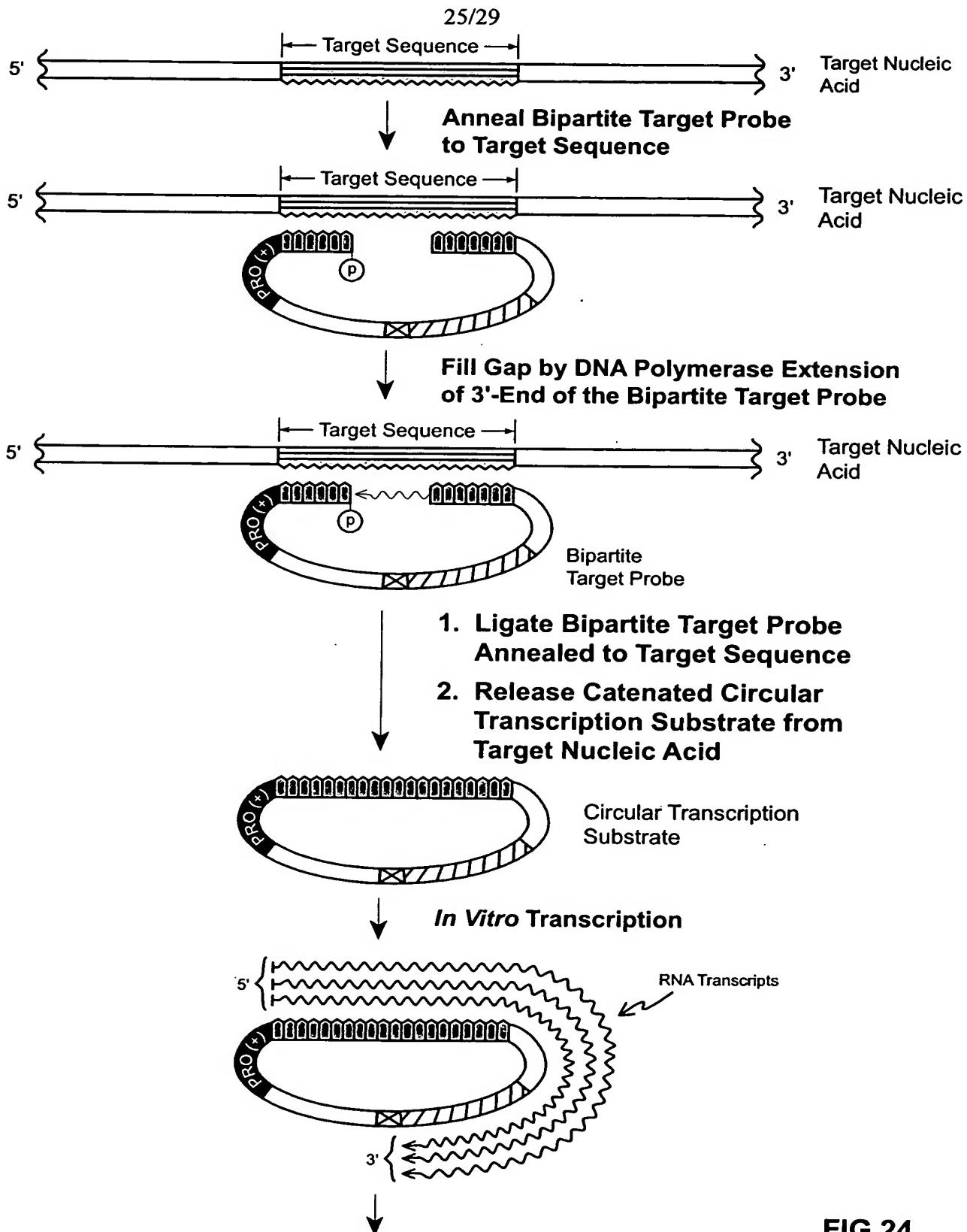


FIG 24

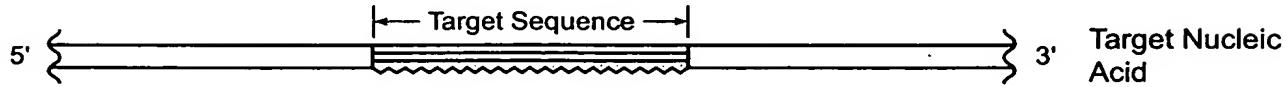
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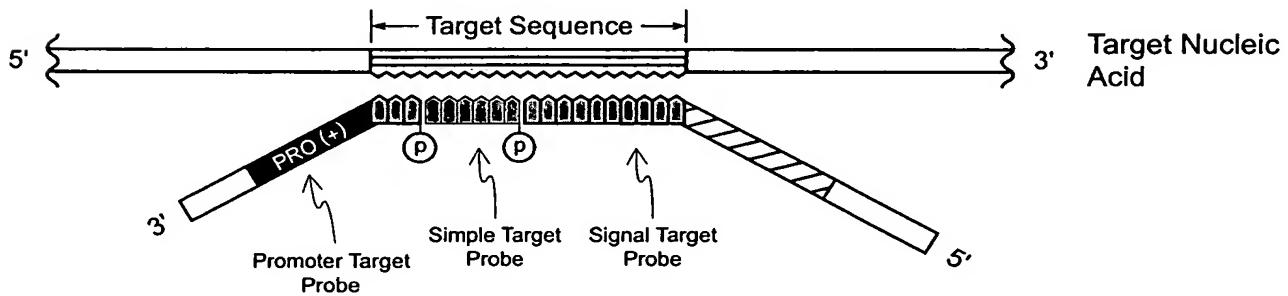
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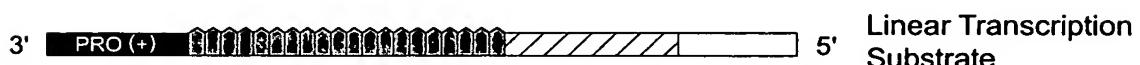
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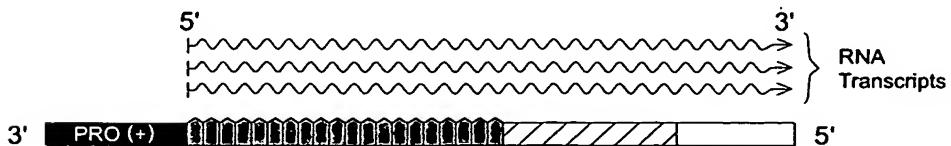
Anneal Monopartite Target Probes to Target Sequence



Ligate Target Probes Annealed to Target Sequence



In Vitro Transcription



Detect RNA Transcription Products Directly or Indirectly

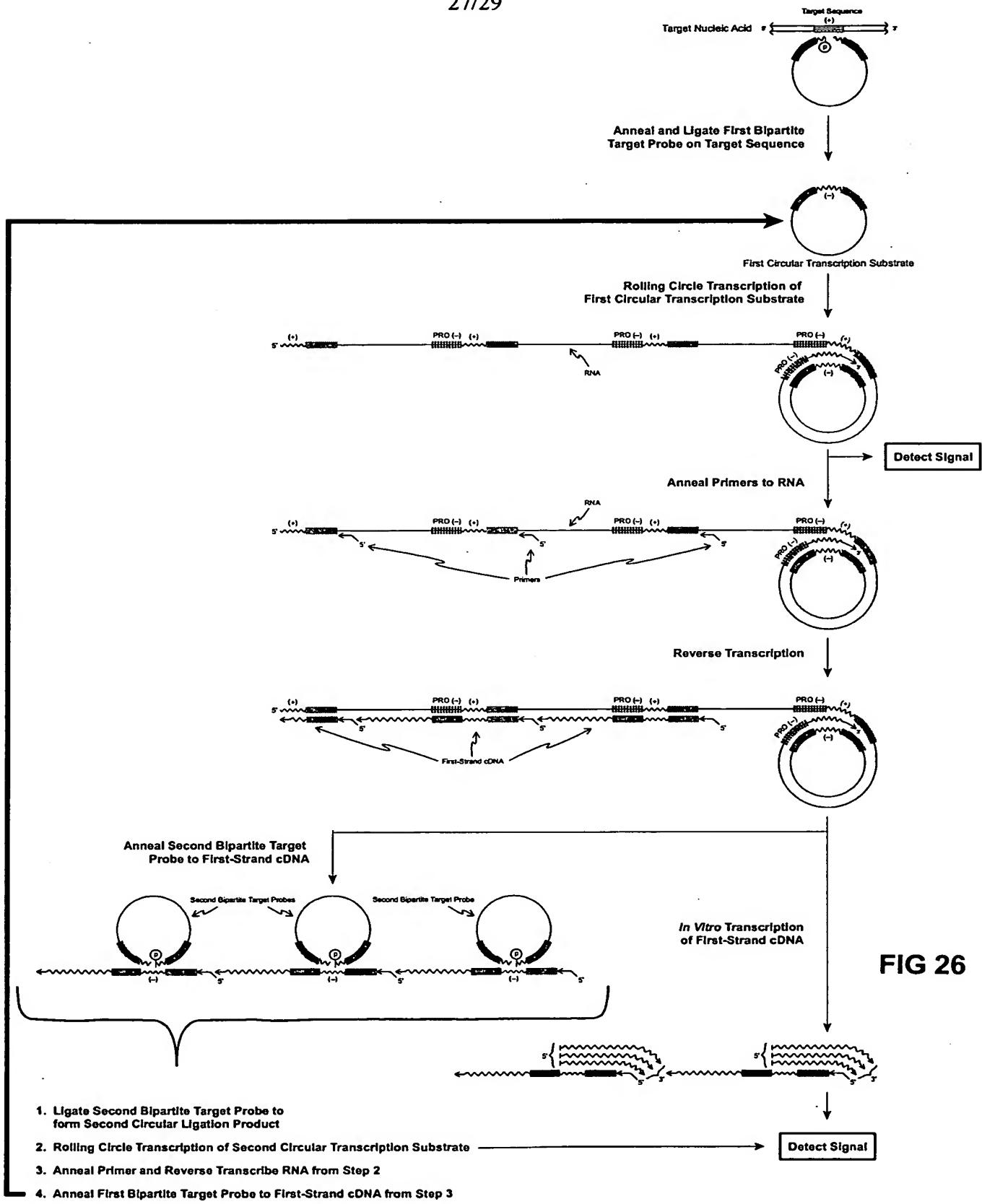
FIG 25

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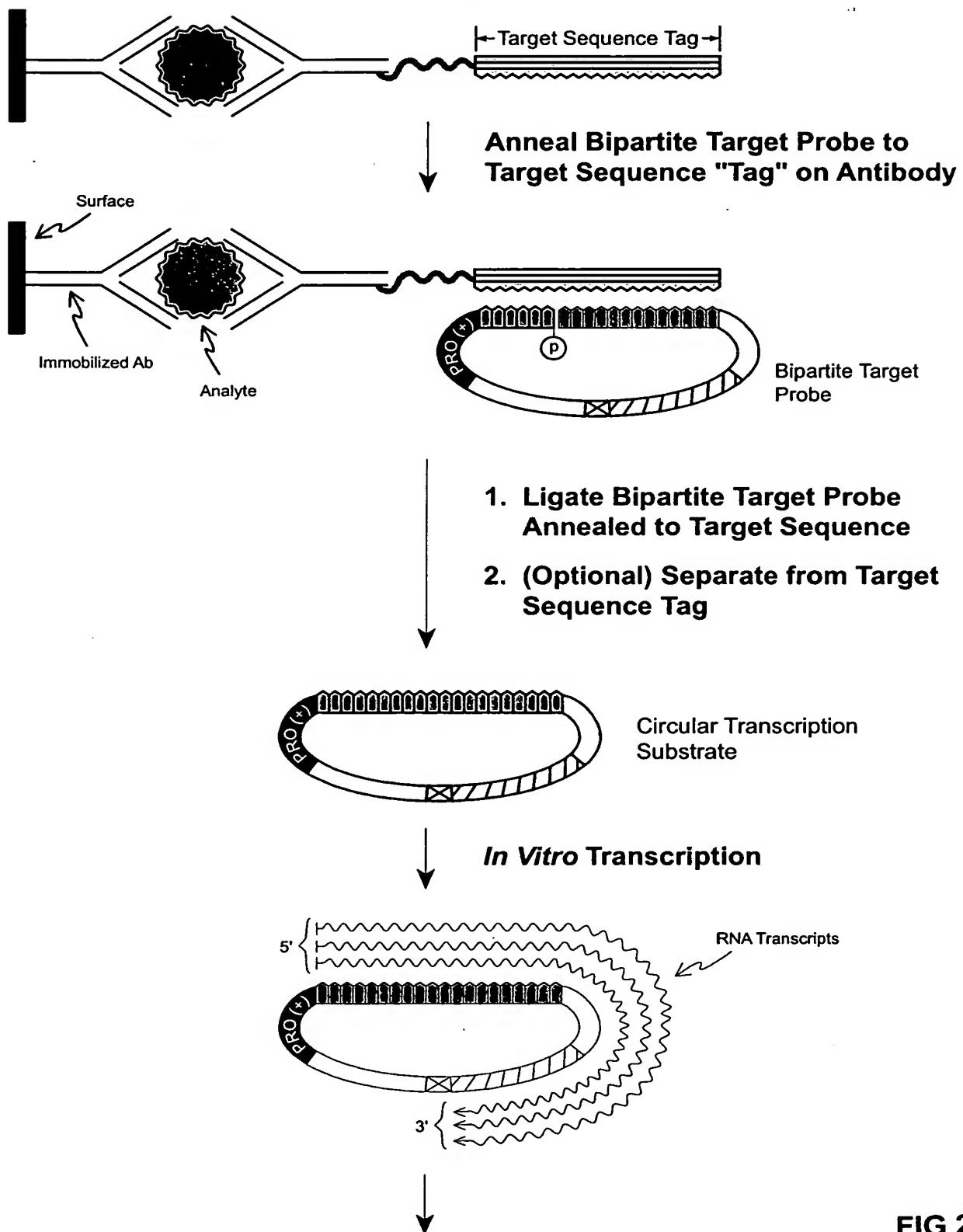
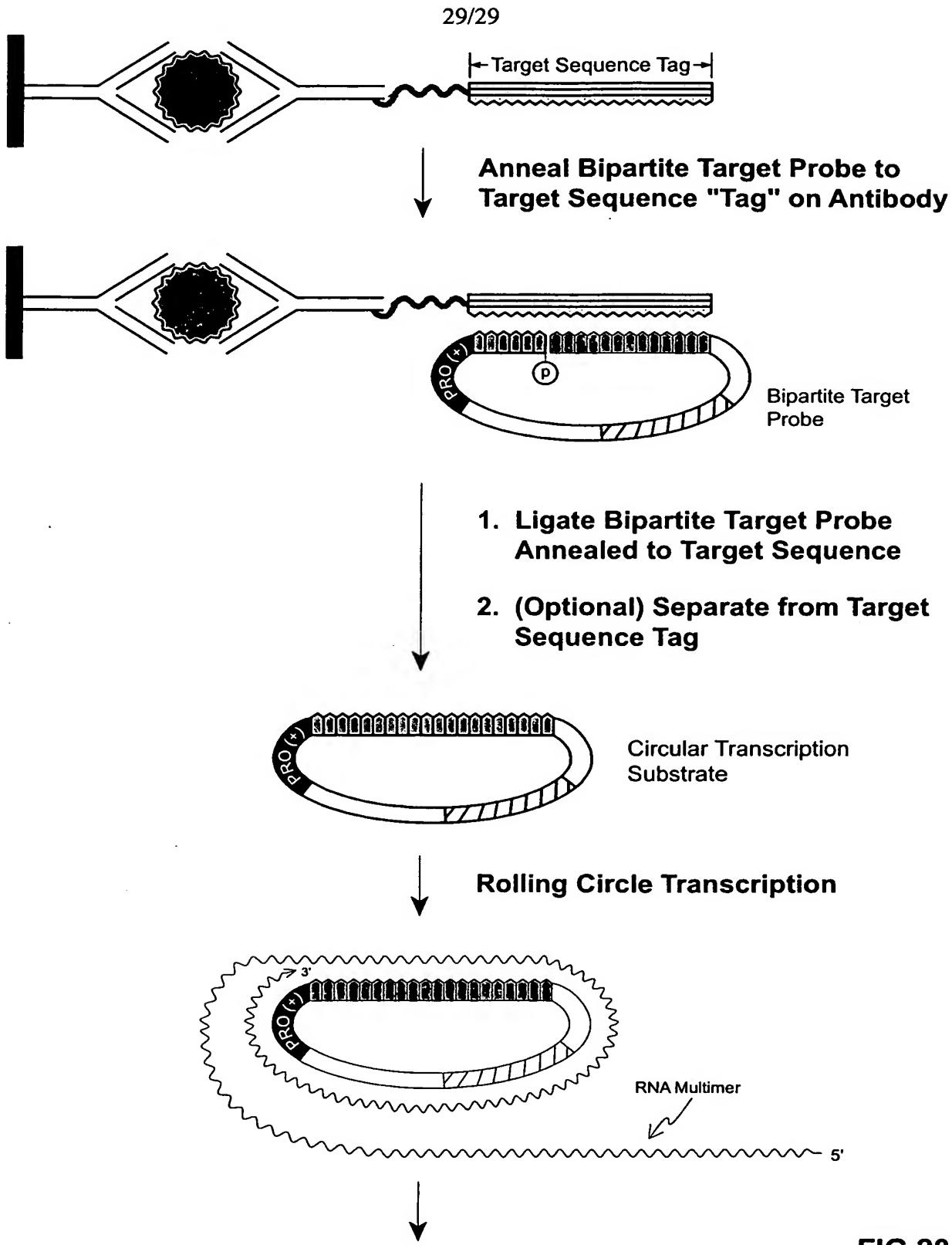


FIG 27

Detect RNA Transcription Products Directly or Indirectly

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Detect RNA Transcription Products Directly or Indirectly

FIG 28